



In the Claims:

Claims 1 – 51 (Canceled).

Claim 52. (NEW) A method for the measurement of biological ligand association with an insoluble surface, comprising:

- [a] applying a labeled ligand, comprising a biological ligand possessing a conjugate specifically recognizable by a detectable group of agents, to a known amount of said surface, in the presence or absence of an unlabeled ligand, comprising of same said biological ligand which does not possess said conjugate, and,
- [b] waiting for a period of time, and,
- [c] removing the non-surface associated labeled ligand and the non-surface associated unlabeled ligand from the surface environment, and
- [d] solubilizing the surface-associated labeled ligand and the surface-associated unlabeled ligand; with or without disruption of said surface, thereby producing a solubilized surface and ligand mixture, and,
- [e] preparing a labeled ligand standard series, comprising a series of formulations of known amounts of the labeled ligand, and,
- [f] optionally separating said solubilized surface and ligand mixture, thereby producing a separated solubilized surface and ligand mixture; while concomitantly separating, in parallel, said labeled ligand standard series, thereby producing a separated ligand standard series, and,

[g] immobilizing onto a support, 1). said solubilized surface and ligand mixture, and said labeled ligand standard series; or, 2). said separated solubilized surface and ligand mixture, and said separated ligand standard series and,

[h] detecting all the support-associated labeled ligand, by applying to said support the detectable specific conjugate recognizing group of agents, thereby producing a signal correlating with the amount of labeled ligand present on the support, and,

[i] determining the amount of the support-associated previously surface-associated said labeled ligand, by comparing its said signal to the signals obtained from the support-associated labeled ligand standard series and,

[j] using the information from [a] and [i] to determine the amount of said labeled ligand originally associated with each said surface amount.

whereby the use of radiolabeled ligand is avoided and,

whereby the sensitive detecting of said labeled ligand is afforded by the use of the conjugate recognizing agents and,

whereby the support position of said signal arising from the separated previously surface associated labeled ligand is verified by comparing it to the support position of said signals arising from said separated labeled ligand standard series and,

whereby the signals arising from previously surface associated samples containing labeled and unlabeled ligand, are compared to the signals arising from previously surface associated samples containing labeled ligand only, thereby ascertaining competition for the surface association between said labeled ligand and said unlabeled ligand, thus verifying specific labeled ligand surface association.

Claim 53. (NEW) The method of claim 52, wherein said surface includes biological cells.

Claim 54. (NEW) The method of claim 52, wherein said conjugate includes entities which are specifically recognized by an antibody.

Claim 55. (NEW) The method of claim 52, wherein said conjugate includes fluorescent labels.

Claim 56. (NEW) The method of claim 56, wherein said conjugate is selected from the group consisting essentially of, fluorescein, biotin, rhodamine, and digoxigenin.

Claim 57. (NEW) The method of claim 52, wherein said biological ligand is a biological factor, a protein, DNA, or an oligonucleotide.

Claim 58. (NEW) The method of claim 57, wherein said protein is selected from the group consisting essentially of transferrin, concanavalin A, avidin, annexin V, and insulin.

Claim 59. (NEW) The method of claim 52, wherein said separating method includes electrophoresis.

Claim 60. (NEW) The method of claim 59 wherein said electrophoresis method is selected from the group consisting essentially of sodium dodecyl sulfate polyacrylamide electrophoresis, electrophoresis according to Schagger Von Jagow, and agarose electrophoresis.

Claim 61. (NEW) The method of claim 52, wherein said immobilization method includes blotting.

Claim 62. (NEW) The method of claim 61, wherein said blotting method is selected from the group consisting essentially of dot blotting, slot blotting, or western blotting.

Claim 63. (NEW) The method of claim 52, wherein said immobilization support includes protein binding materials.

Claim 64. (NEW) The method of claim 52, wherein said immobilization support includes nucleic acid binding materials.

Claim 65. (NEW) The method of claim 52, wherein said immobilization support includes blotting membranes.

Claim 66. (NEW) The method of claim 64, wherein said blotting membrane is selected from the group consisting essentially of nitrocellulose, polyvinylidenedifluoride, or nylon.

Claim 67. (NEW) The method of claim 52, wherein said detecting of said immobilization support associated labeled ligand, further comprises applying to said support said detectable group of agents in a sequence, wherein said sequence is comprising:

- [a] applying to said immobilization support a blocking solution, thereby occupying all available sites on the support and,**
- [b] applying to said immobilization support a first detection agent which specifically recognizes the support-associated labeled ligand, and,**
- [c] removing of all the unreacted detection agent from the immobilization support and,**
- [d] applying to said immobilization support a subsequent detection agent which specifically recognizes the previous detection agent and,**

[e] removing of all the unreacted subsequent detection agent from the
immobilization support and,

[f] repeating [d] and [e] until all agents in the sequence are utilized;

wherein the final detection agent used in said sequence further comprises the
detection agent which possesses a detectable moiety, and; wherein the number
of said subsequent detection agents is unlimited, and; wherein if said number
of subsequent agents is zero, said first detection agent comprises the entire
sequence, thereby said first detection agent comprises the final detection
agent, and thereby said first detection agent includes said detectable moiety,
and; wherein determining the amount of immobilization support associated
said final detection agent is performed by colorimetric, luminescent, chemical,
or physical based detecting of said detectable moiety on said final detection
agent, thereby producing said signal of claim 52.

Whereby increased sensitivity is afforded by the using of multiple
subsequent detection agents.

Claim 68. (NEW) The method of claim 67, wherein said first detection agent includes an
antibody to said labeled ligand.

Claim 69. (NEW) The method of claim 67, wherein said subsequent detection agent
includes an antibody to the previous support-associated detection agent.

Claim 70. (NEW) The method of claim 67 wherein said blocking solution comprises a buffer solution containing a non-ionic detergent and a blocking reagent, wherein said blocking reagent is selected from a group consisting essentially of non-fat dry milk, and gelatin.

Claim 71. (NEW) The method of claim 67, wherein the said final agent detectable moiety includes conjugated enzymes.

Claim 72. (NEW) The method of claim 67, wherein said conjugated enzyme includes horseradish peroxidase.

Claim 73. (NEW) The method of claim 52, wherein exposing of said surface to the labeled ligand in varied conditions, whereby said insoluble surface is capable of deformation, is used to measure said surface labeled ligand binding or internalization.

Claim 74. (NEW) The method of claim 52, wherein the determining of the specific binding of the labeled ligand to said surface is performed by comparing the signals arising from the previously surface associated samples containing labeled and unlabeled ligand, to the signals arising from the previously surface associated samples containing labeled ligand only, thus ascertaining the competition for surface association between labeled and unlabeled ligand.